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ARTICLE

Efficacy of *Phytoseiulus persimilis* and *Amblyseius swirskii* for integrated pest management for greenhouse cucumbers under Mediterranean environmental conditions

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Abstract

The greenhouse cucumber pests, *Bemisia tabaci* (Hemiptera: Aleyrodidae), *Frankliniella occidentalis* (Thysanoptera: Thripidae), and *Tetranychus urticae* (Acari: Tetranychidae), are major threats to the production of greenhouse cucumbers (Cucurbitaceae) in Lebanon. The development of insecticide resistance by these pests has prompted the use of alternative and sustainable pest management strategies. In this study, we used integrated pest management strategies, including the release of the biological control agents, *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae) and *Phytoseiulus persimilis* Athias-Henriot (Mesostigmata: Phytoseiidae), to control whitefly, thrips, and two-spotted spider mite populations on greenhouse cucumber plants in two commercial production sites (sites A and B). We also compared the efficacy of pest population suppression using the integrated pest management strategy with that of chemical pest control. Our results show that biological control effectively maintains the cucumber pest populations below the economic threshold when coupled with additional integrated pest management measures. In addition, we show that biological control agents were equally or more effective in pest population suppression compared to eight and 12 insecticidal and acaricidal sprays performed in the control greenhouses at sites A and B, respectively. Altogether, our results show the efficacy of adopting integrated pest management and biological control for pest population suppression in greenhouse cucumber production under Mediterranean environmental conditions.

Introduction

Integrated pest management is a technique widely used to reduce pest populations by integrating diverse strategies, including biological control agents (van Lenteren and Woets 1988; Peshin and Dhawan 2009; Hoy 2012; Naranjo *et al.* 2015). Biological control helps to prevent the development of insecticide resistance and minimises the impact of insecticides on the environment and human health (van Lenteren and Woets 1988; Barzman *et al.* 2015). The

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numerous benefits of using biological control agents over chemical pest control have driven interest in their application in diverse pest control strategies.

Although the greenhouse environment favours optimal cucumber (Cucurbitaceae) production, it also favours the rapid development of insect and mite populations (Messelink *et al.* 2020). Among cucumber arthropod pests, whiteflies, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), and two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), cause significant damage to the cucumber host directly through feeding on plant sap and indirectly through transmitting viral diseases (McClanahan 1970; Rosenheim *et al.* 1990; Gaum *et al.* 1994; Hao *et al.* 2002; Park and Lee 2002, 2005; Jones 2003). The primary strategy to control these cucumber pests has been the application of insecticides and acaricides. However, the over-reliance on and misuse of these chemical pest control agents have promoted insecticide and acaricide resistance (Gorman *et al.* 2002; Van Leeuwen *et al.* 2010; Gao *et al.* 2012; Lebedev *et al.* 2013; Cloyd 2016; Horowitz *et al.* 2020). Alternative pest control strategies are required to maintain the whitefly, thrips, and two-spotted spider mite populations below damaging levels on cucumber plants.

Amblyseius swirskii Athias-Henriot (Mesostigmata: Phytoseiidae), a generalist predatory mite originating from the east Mediterranean coast (Athias-Henriot 1962; Calvo *et al.* 2015; Demite *et al.* 2015), is widely used in biological control strategies on numerous ornamental and vegetable crops, including solanaceous and cucurbit crops (Calvo *et al.* 2015). *Amblyseius swirskii* efficiently controls diverse arthropod pest populations, including whiteflies, thrips, and to a lesser extent, several mite species (Cock *et al.* 2010; van Maanen *et al.* 2010; van Lenteren 2012; Calvo *et al.* 2015). Laboratory and greenhouse studies report the effective suppression of whitefly and thrips populations either separately or during simultaneous infestation on cucumber leaves by adult *A. swirskii* mites (Nomikou *et al.* 2001, 2002; Messelink *et al.* 2005, 2006; Calvo *et al.* 2011). However, knowledge on the efficacy of whitefly and thrips population control by *A. swirskii* under commercial greenhouse production conditions in Lebanon remains lacking.

Although *A. swirskii* adults prey on two-spotted red spider mite eggs and nymphs, their main host preference remains whiteflies and thrips (Xu and Enkegaard 2010; Calvo *et al.* 2015). This preference necessitates the use of an additional natural enemy, the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Mesostigmata: Phytoseiidae). *Phytoseiulus persimilis* is a specialised predator of *Tetranychus* sp., including the two-spotted spider mites (McMurtry *et al.* 2013). Since the 1960s, *P. persimilis* has been used widely for the efficient control of two-spotted spider mite populations in cucumber plants under greenhouse and laboratory conditions (Masoud 2007; van Houten *et al.* 2007b; Gillian 2008; Gontijo *et al.* 2010; McMurtry *et al.* 2013; Fathipour *et al.* 2017). This predatory mite species is characterised by a high population density when fed on *T. urticae* eggs and nymphs (Escudero and Ferragut 2005; Moghadasi et al. 2016). Furthermore, studies have determined *P. persimilis* to tolerate increasing temperatures and humidity levels, rendering it suitable for greenhouse environments (Mori and Chant 1966; Stenseth 1979; Skirvin and Fenlon 2003). Therefore, the ability of *P. persimilis* to reproduce while feeding on different *T. uritcae* life stages and to tolerate increased temperatures and humidity makes it a desirable candidate for two-spotted spider mite management on cucumber plants under greenhouse conditions. However, the simultaneous introduction of different predatory mites can lead to a negative outcome due to intraguild predation, whereby competition for the same food can lead to cannibalism among species (Polis 1981; Polis *et al.* 1989). Few studies have shown intraguild predation by *A. swirskii* mites on *P. persimilis* nymphs, larvae, and eggs in the absence of an alternative and preferred food source (Haghani *et al.* 2015; Maleknia *et al.* 2016). However, a study conducted on sweet-pepper plants (Solanaceae) showed successful control of *T. urticae* by *P. persimilis* in the presence of *A. swirskii* mites (van Houten *et al.* 2007a). This suggests that when preferred prey are available, the introduction of two predatory mite species to control greenhouse pests may be possible.

The overall objective of this study was to evaluate the efficacy of integrated pest management strategies that mainly rely on the release of *A. swirskii* and *P. persimilis* and are complemented, when needed, by the application of pesticides that are relatively safe to predatory mites for the control of whiteflies, thrips, and two-spotted spider mites on greenhouse cucumbers, as compared to the efficacy of routine chemical pest control strategies that are commonly followed by commercial cucumber growers in Lebanon. Pesticide applications in the integrated pest management greenhouses were determined by using published economic thresholds for each pest and the expected arrival of predatory mites. Economic threshold is defined as the insect population density at which control measures should be taken to prevent the pest population from reaching economically damaging levels (Higley and Pedigo 1996; Costa *et al.* 2019). The economic threshold varies for each pest, being 4.6 adult whiteflies per leaf (Shen *et al.* 2005), 1.3 adult thrips per leaf (Steiner 1990), and 3–5 adult thrips per flower (Shipp *et al.* 2000). Although the economic threshold of two-spotted spider mites on cucumber leaves has not yet been reported, a population of two two-spotted spider mite adults per leaf at the seventh- or eighth-leaf stage was shown to cause a 23.8% loss in cucumber yield (Atanassov 1997). This value was used as an indicator of acaricide application in the integrated pest management greenhouses.

Materials and methods

Greenhouses

Experiments were conducted at site A in Tamich, Mount Lebanon, Lebanon at an altitude of 250 m, and at site B in Zahrani, Southern Lebanon, at 5 m. At each site, two polyethylene plastic–covered greenhouses equipped with 50-mesh insect-proof nets were randomly selected. The area of each greenhouse at site A was 414 m^2, whereas that at site B was 320 m^2. This study was conducted in greenhouses provided by commercial vegetable growers, and only two greenhouses from each location were provided for our experiments. At each location, one greenhouse served as a control, where conventional pest management techniques were followed; in the other greenhouse, integrated pest management practices were implemented, with multiple natural enemy releases. Access to the greenhouses was granted through double doors. Daily temperature (°C) and relative humidity (%) were recorded at 15-minute intervals in the integrated pest management greenhouses at both sites, using HOBO data loggers (Ebro, New York, New York, United States of America).

Plants and cultural practices

In the integrated pest management greenhouses, plant debris from the previous growing season was removed and burned to minimise disease incidence. Additionally, weeds surrounding and growing inside the greenhouses were removed by hand. In the control greenhouses, one week before transplanting, the polyethylene plastic covers were sprayed with the insecticide Morgan (Lapisa, Mexico; 48% chlorpyrifos ethyl w/v) at the company’s recommended application rate of 2.5 mL/L to eliminate insects in the greenhouses.

Seedlings of cucumber, *Cucumis sativus* Linnaeus, varieties “New sun” and “Mira” were transplanted at a rate of three seedlings per square metre in the greenhouses at sites A and B on 3 November and 19 October 2016, respectively. The integrated pest management and control greenhouses at sites A and B contained 1240 and 960 cucumber seedlings, respectively, transplanted in 10 rows. The farmer at site A used a hydroponic farming method with hydrogen peroxide–disinfected coco-peat as a growth substrate, whereas the farmer at site B adopted a conventional farming method using soil as the growing medium. One week before transplanting the seedlings, three seedlings per pot (diameter: 17 cm; height: 13 cm) of flowering marigolds, *Tagetes patula* (Asteraceae) (Marigold French Hero, Yellow; Ball Seed, West Chicago, Illinois, United States of America), were introduced in the integrated pest management greenhouses at a rate of 1 pot/32 m^2 to attract existing and newly emerging thrips populations (Kasina *et al.* 2006). Before the cucumber seedlings were transplanted, the pots of marigolds were covered with plastic bags and discarded and new uninfested pots of marigolds were introduced. In addition, yellow sticky traps were installed in the integrated pest management greenhouses at a rate of 1 card/16 m^2 to monitor existing whiteflies and thrips. To scout the sticky traps, the integrated pest management greenhouses were divided into quadrants. Five traps were removed – one from each greenhouse quadrant and one from the middle – they were scouted and then replaced by new traps every week.

Predatory mites

Upon consultation with technical advisors from BioBest (Westerlo, Belgium), the predatory mites *A. swirskii* and *P. persimilis* were selected as suitable predatory mites for our experimental conditions. The predatory mites and Nutrimite, an alternative food source for *A. swirskii* that consists of the pollen of the narrow-leaved cattail, *Typha angustifolia* (Typhaceae), were obtained from a commercial supplier (BioBest). *Amblyseius swirskii* was provided in two formats: in cardboard bottles, each of which contained 25 000 individuals (nymphs and young adults); and in 500 slow-release sachets, each of which contained 250 nymphs and young adults (totalling 125 000 individuals), according to the experimental requirements. Both formats were mixed with bran and factitious prey, *Tyrophagus putrescentiae* (Sarcoptiformes: Acaridae), as a food source during transport in cold containers. *Phytoseiulus persimilis* were obtained in plastic bottles, each containing 2000 individuals (young adults), with vermiculite as a carrier (Table 1). All predatory mites were kept at room temperature for 30 minutes before release, per manufacturer's recommendation. *Amblyseius swirskii* mites received in slow-release sachets were hung on the stems of randomly selected cucumber plants, and those received in bottles were sprinkled on randomly selected cucumber leaves. At the beginning of the growing season, the seedlings were dusted with *T. angustifolia* pollen as an alternative food source for *A. swirskii*. *Phytoseiulus persimilis* mites were sprinkled in hot spots – that is, on cucumber leaves infested by two-spotted spider mites. Predatory mite releases and pollen sprays were not performed in the control greenhouses. Throughout the growing season, infestation of the cucumber plants by *B. tabaci*, *F. occidentalis*, and *T. urticae* occurred naturally, without the pests being introduced by artificial means.

Experimental design and sampling

The effect of *A. swirskii* and *P. persimilis* on whiteflies, thrips, and two-spotted spider mites was assessed through four predatory mite releases in the integrated pest management greenhouse at site A and through five predatory mite releases in the integrated pest management greenhouse at site B. Cucumber plants at both sites were scouted weekly for predatory mite and pest populations. Scouting was performed on 50 randomly selected cucumber plants (five cucumber plants per row). Three leaves and three flowers from the top, middle, and lower parts of each selected cucumber plant were randomly chosen and scouted, totalling 150 leaves and 150 flowers per greenhouse per week for 20 weeks in the control greenhouses and for 25 and 22 weeks in the integrated pest management greenhouses at sites A and B, respectively. Additionally, marigold flowers were scouted weekly for infestation by adult thrips. Highly infested marigold plants and cucumber leaves were removed from the integrated pest management greenhouses and burned, with new, uninfested marigolds then introduced.

Table 1. Predatory mite (*Amblyseius swirskii* and *Phytoseiulus persimilis*) release dates and rates in the integrated pest management greenhouses at sites A and B.

Site A			Site B		
Date (week)	Predatory mites	Rate (mites or pollen/m^2)	Date (week)	Predatory mites	Rate (mites or pollen/m^2)
22 November 2016 (3)	*A. swirskii* system bottle (25 000 mites)	60	4 November 2016 (2)	*A. swirskii* system bottle (25 000 mites)	78
	Nutrimite	0.15 g		Nutrimite	0.15 g
14 December 2016 (6)	*A. swirskii* breeding system (100 sachets)	60	22 November 2016 (5)	*A. swirskii* breeding system (100 sachets)	78
	P. persimilis 2 bottles (2000 mite/bottle)	10			
23 February 2017 (17)	*A. swirskii* breeding system (100 sachets)	60	14 December 2016 (8)	*A. swirskii* breeding system (100 sachets)	78
	P. persimilis 2 bottles (2000 mites/bottle)	10		*P. persimilis* 2 bottles (2000 mite/bottle)	12.5
31 March 2017 (22)	*A. swirskii* system bottle (1/2 bottle) (25 000 mites)	60	22 February 2017 (17)	*A. swirskii* breeding system (100 sachets)	78
	P. persimilis 6 bottles (2000 mites/bottle)	29		*P. persimilis* 2 bottles (2000 mites/bottle)	12.5
			31 March 2017 (21)	*P. persimilis* 2 bottles (2000 mites/bottle)	31 March 2017

The number and rate of releases depended on the environmental conditions and pest infestation level. See Table 1 for details of the predatory mite release rates and dates. Based on pest population–monitoring results, the first releases of *A. swirskii* mites at sites A and B were on weeks 3 and 2 of the experimental period, respectively. However, releases of *P. persimilis* mites were delayed until weeks 6 and 8 at sites A and B, respectively, due to the absence of two-spotted spider mites early in the growing season.

Pesticide applications

In the control greenhouses, the farmers maintained their routine insect and disease management strategies. However, in the integrated pest management greenhouses, data sheets published by Koppert (Berkel en Rodenrijs, The Netherlands) and BioBest were used to select pesticides with minimal side effects on the predatory mites. See Supplementary material, Tables S1 and S2 for details of the applied pesticides, including rates and dates of application, active ingredients, and target pests, for both greenhouses at sites A and B.

Statistical analysis

The weekly insect scouting data from 150 leaves and 150 flowers were assessed for non-normality using the Shapiro–Wilk normality test. The effects of the predatory mites on whitefly, thrips, and spider mite populations were compared to the pests' populations in the control greenhouses on the mean weekly scouting data (weeks 1–20) of 150 leaves and flowers using the nonparametric Mann–Whitney *U* test and parametric Welch's *t*-test. Spearman's Rho was used to describe the correlation between the mean populations of *A. swirskii,* whitefly, and thrips, *P. persimilis* and two-spotted spider mites, and *P. persimilis* and *A. swirskii* from 150 leaves per week until the experiment was ended in the integrated pest management greenhouses (at week 25 at site A and at week 22 at site B). The strength of the relationship between predatory mites and the pest populations was determined using the correlation coefficient *r* and was described as no correlation (0.0), weak (0.1–0.3), moderate (0.4–0.6), and strong (0.7–1.0; Akoglu *et al.* 2018). In addition, the mean whitefly adult population from five yellow sticky traps per week was used to assess possible adult migration into the integrated pest management greenhouses from the surrounding environment. All the statistical analyses were performed using GraphPad prism, version 8.4.3.

Results

Ambient conditions

In site A, the mean weekly temperature varied from 14.2 °C in week 3 to 18.3 °C in week 20 (minimum and maximum mean temperatures being 5.8 °C and 26.2 °C, respectively). The average relative humidity ranged from 45 to 80% (Fig. 1A). In site B, the mean weekly temperature varied from 24.3 °C in week 3 to 22.6 °C in week 20 (minimum and maximum mean temperatures being 12.18 °C and 24.3 °C, respectively), and the average relative humidity ranged from 60 to 80% (Fig. 1B).

Whitefly management

Site A. In the site A control greenhouse, five insecticide applications maintained the whitefly nymph and adult populations below the economic threshold throughout the growing period (Fig. 2A). In the integrated pest management greenhouse, the whitefly adult population remained below the economic threshold of 4.6 whitefly adults per leaf (Shen *et al.* 2005) during the 23-week growing period. Similarly, without application of insecticides targeting

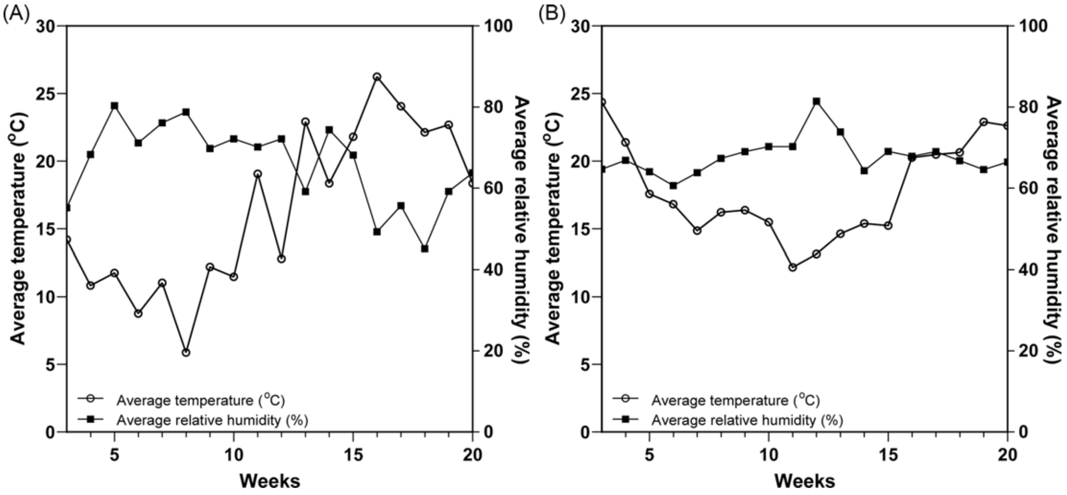

Fig. 1. Mean temperature (°C) and relative humidity (%) under the integrated pest management greenhouse conditions per week from early winter 2016 until spring 2017. **A**, Mean temperature and relative humidity in the integrated pest-managed greenhouse at site A; **B**, mean temperature and relative humidity in the integrated pest-managed greenhouse at site B.

whiteflies, whitefly nymph numbers remained below 1.5 insects per leaf during the entire growing period (Fig. 2B). In the last two weeks of the experiment, a peak of eight whitefly adults per leaf was recorded. In addition, the mean number of adult whiteflies recorded on sticky cards (9.4 ± 1.2) was more than 30 times higher than the mean number of adult whiteflies recorded on cucumber leaves (0.320 ± 0.076), suggesting whitefly adult migration from the surrounding environment. A significant moderate positive correlation was recorded between the whitefly adult and *A. swirskii* populations ($r = 0.452$; $P = 0.023$; Table 3), whereas a nonsignificant weak positive correlation was recorded between whitefly nymphs and *A. swirskii* populations ($r = 0.167$, $P = 0.424$). The mean numbers of whitefly nymphs and adults on cucumber leaves in the integrated pest management greenhouse did not significantly differ compared to the nymph and adult populations in the control greenhouse ($U = 174.5$, $P = 0.268$; $U = 182.5$, $P = 0.644$; Table 2).

Site B. At site B, the whitefly nymph and adult populations were maintained below the economic threshold in both the integrated pest management and control greenhouses. With 12 whitefly-targeting insecticide applications in the control greenhouse, the peak was two whitefly adults per leaf (Fig. 2C). In the integrated pest management greenhouse, where no whitefly-targeting insecticide had been applied, the peak of whitefly adults on cucumber leaves was 2.7 adults (Fig. 2D). Similar to site A, the mean number of adult whiteflies recorded on sticky cards (8.2 ± 0.840) was more than 30 times higher than the mean number of the adult flies recorded on cucumber leaves (0.713 ± 0.178). A significant moderate positive correlation was recorded between the whitefly adult and *A. swirskii* populations ($r = 0.585$, $P = 0.0042$; Table 3). However, the moderate positive correlation between the nymph population with that of the predatory mite was not significant ($r = 0.371$; $P = 0.089$; Table 3). The mean number of whitefly nymphs, although below the economic threshold, was significantly higher in the integrated pest management greenhouse ($U = 131$, $P = 0.0436$). However, the mean number of whitefly adults did not significantly differ between the integrated pest management and control greenhouses ($U = 159.5$, $P = 0.279$; Table 2).

Thrips management

Site A. At site A, in the control greenhouse, the application of five thrips-targeting insecticidal sprays contributed to the decline of the thrips population on cucumber leaves and flowers to below

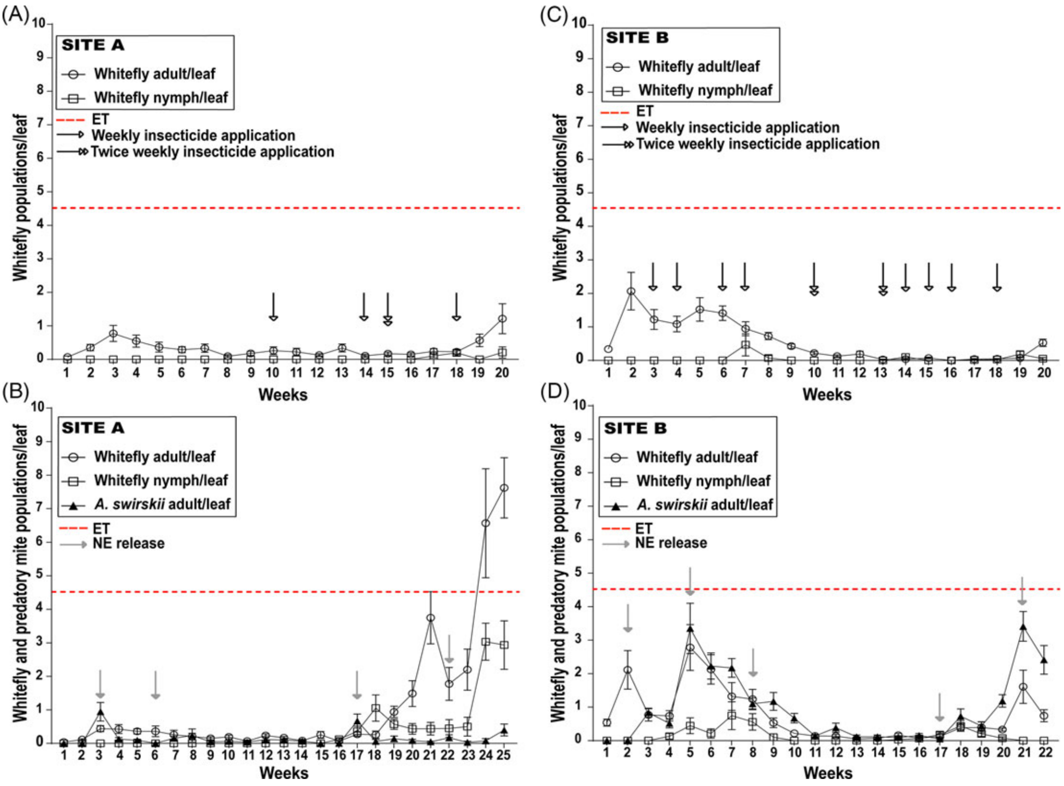

Fig. 2. The average number of whitefly nymphs and adults and *Amblyseius swirskii* adults on cucumber leaves in the integrated pest management and control greenhouses at sites A and B. **A**, Whitefly nymph and adult populations in the control greenhouse at site A; **B**, whitefly nymph and adult and *A. swirskii* populations per cucumber leaf in the integrated pest-managed greenhouse at site A; **C**, whitefly nymph and adult populations in the control greenhouse at site B; **D**, whitefly nymph and adult and *A. swirskii* adult populations on per cucumber leaf in the integrated pest-managed greenhouse at site B. The error bars indicate the standard error of mean. Natural enemy releases (NE; grey arrows), weekly whitefly-targeting insecticide sprays (black arrows), and economic threshold (ET; dashed red line).

the economic threshold of 1.3 thrips adults per leaf (Steiner 1990) and 3–5 thrips per flower (Shipp *et al.* 2000). The thrips population was maintained below the economic threshold for up to 10 weeks, followed by an increase in thrips numbers to a peak of three thrips adults per leaf (Fig. 3A). In the integrated pest management greenhouse, four *A. swirskii* releases maintained the thrips population below the economic threshold throughout the growing period (Fig. 3B). Peaks in *A. swirskii* numbers were recorded a week following each release. A weak nonsignificant positive correlation occurred between the thrips and *A. swirskii* populations ($r = 0.209$; $P = 0.316$; Table 3). Although the thrips population remained below the economic threshold during most of the growing season, the mean number of thrips recorded per cucumber leaf and flower was significantly higher in the control greenhouse compared to the corresponding mean number recorded in the integrated pest management greenhouse ($U = 61$, $P < 0.0001$; $U = 103.5$, $P = 0.007$; Table 2).

Site B. In the site B control greenhouse, 12 insecticidal sprays targeting thrips kept the thrips population on cucumber leaves and flowers below the economic threshold throughout the growing season (Fig. 3C). In the integrated pest management greenhouse, a thrips count of 1.5 thrips per leaf was recorded starting week 11, but the population on cucumber flowers remained below the economic threshold. As temperatures increased after week 11, the thrips

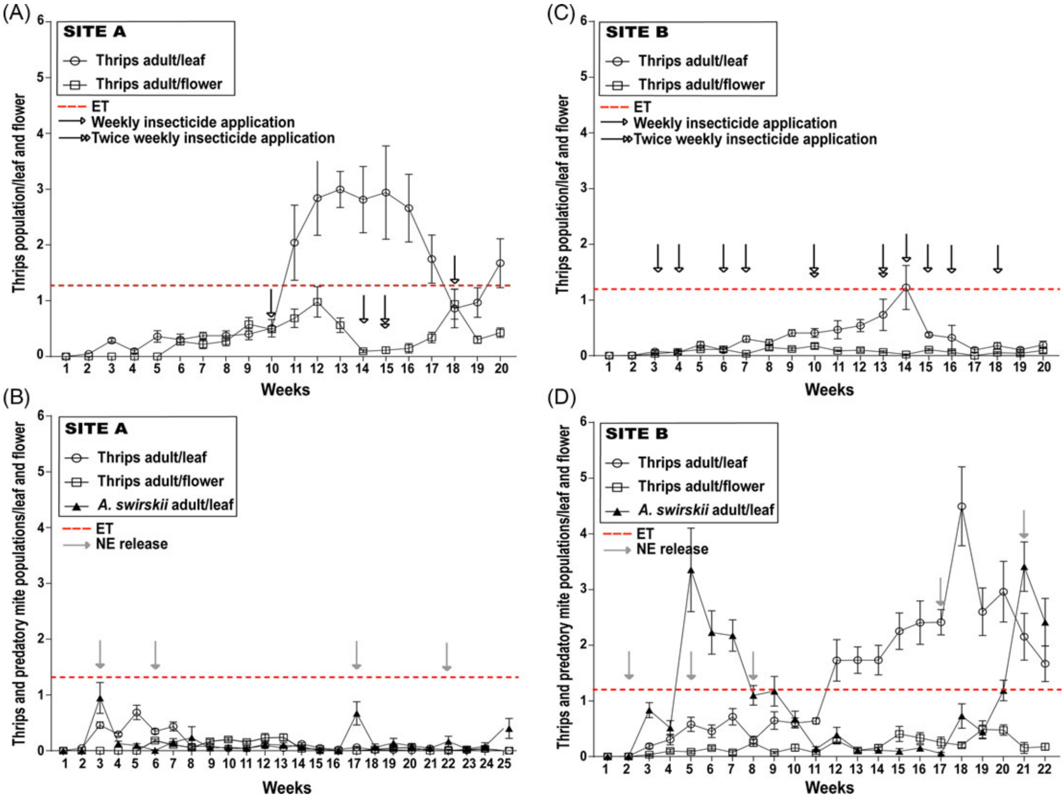

Fig. 3. The average number of adult thrips and *Amblyseius swirskii* on cucumber leaves and flowers in the integrated pest management and control greenhouses at sites A and B. **A**, Thrips population per cucumber leaf and flower in the control greenhouse at site A; **B**, thrips and *A. swirskii* populations per cucumber leaf and thrips per cucumber flower in the integrated pest-managed greenhouse at site A; **C**, thrips population per cucumber leaf and flower in the control greenhouse at site B; **D**, thrips and *A. swirskii* populations per cucumber leaf and thrips per cucumber flower in the integrated pest-managed greenhouse at site B. The error bars indicate the standard error of mean. Natural enemy releases (NE; grey arrows), weekly thrips-targeting insecticide sprays (black arrows), and economic threshold (ET; dashed red line).

population peaked beyond the economic threshold, reaching a maximum of 4.5 thrips adults per leaf. A new introduction of *A. swirskii* on week 17 led to the gradual decline in the thrips population. By the end of the growing season, the thrips population on cucumber leaves was declining towards the economic threshold, whereas the *A. swirskii* population had increased to 3.5 predatory mites per leaf (Fig. 3D). There was no evidence of a correlation between the populations of thrips on cucumber leaves and flowers and that of *A. swirskii* ($r = 0.019$; $P = 0.934$, and $r = 0.031$; $P = 0.890$; Table 3). The mean number of thrips on cucumber leaves and flowers was significantly higher in the integrated pest management greenhouse than in the control greenhouse ($U = 81$, $P = 0.0009$; $t = 3.235$, $P = 0.0036$, and Welch's $t = 3.23$; $P = 0.0036$; Table 2).

Two-spotted spider mite management

Site A. In the integrated pest management and control greenhouses at site A, two-spotted spider mite numbers were below two mites per leaf until weeks 15 and 16. In the control greenhouse, despite seven acaricide sprays, the two-spotted spider mite population increased

Table 2. The mean number of insects and spider mites ± standard error of mean (SEM) in the integrated pest management (IPM) and control greenhouses at both study sites. The nonparametric Mann–Whitney *U* test was used to test for significant difference between the treatment groups.

Study site	Management programme	Mean thrips/leaf ± SEM*	Mean thrips/flower ± SEM	Mean whitefly adults/leaf ± SEM	Mean whitefly nymphs/leaf ± SEM	Mean MITES/LEAF ± SEM
Site A	IPM	0.121 ± 0.036	0.063 ± 0.016	0.320 ± 0.076	0.127 ± 0.062	1.478 ± 0.532
	Control	1.214 ± 0.251	0.321 ± 0.068	0.331 ± 0.061	0.025 ± 0.014	3.348 ± 1.290
	Mann–Whitney *U*	65.5	121	182.5	174.5	175.5
	P-value	< 0.0001*	0.0022*	0.644	0.268	0.516
Site B	IPM	1.34 ± 0.272	0.185 ± 0.033	0.713 ± 0.178	0.159 ± 0.049	0.706 ± 0.183
	Control	0.301 ± 0.065	0.071 ± 0.011	0.556 ± 0.138	0.046 ± 0.024	0.366 ± 0.104
	Mann–Whitney *U*	81	–	159.5	131	161
	Welch's *t*-test	–	3.235	–	–	–
	P-value	< 0.0009*	0.0036*	0.2796	0.0436*	0.2951

*Statistical significance at $P < 0.05$.

Table 3. Spearman correlation test between the cucumber pests and predatory mites (*Amblyseius swirskii* and *Phytoseiulus persimilis*) in the integrated pest management greenhouses at both study sites.

	Site A				Site B			
	A. swirskii		*P. persimilis*		*A. swirskii*		*P. persimilis*	
Insects/spider mite	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Whitefly adults	0.452	0.023*			0.585	0.004*		
Whitefly nymphs	0.167	0.424			0.371	0.089		
Thrips adults/leaf	0.209	0.316			0.019	0.934		
Thrips adults/flower	−0.056	0.789			0.031	0.890		
Two-spotted spider mites			0.649	0.0004*			0.788	< 0.0001*
Predatory mite								
P. persimilis	0.391	0.585			0.010	0.949		

*Statistical significance at $P < 0.05$.

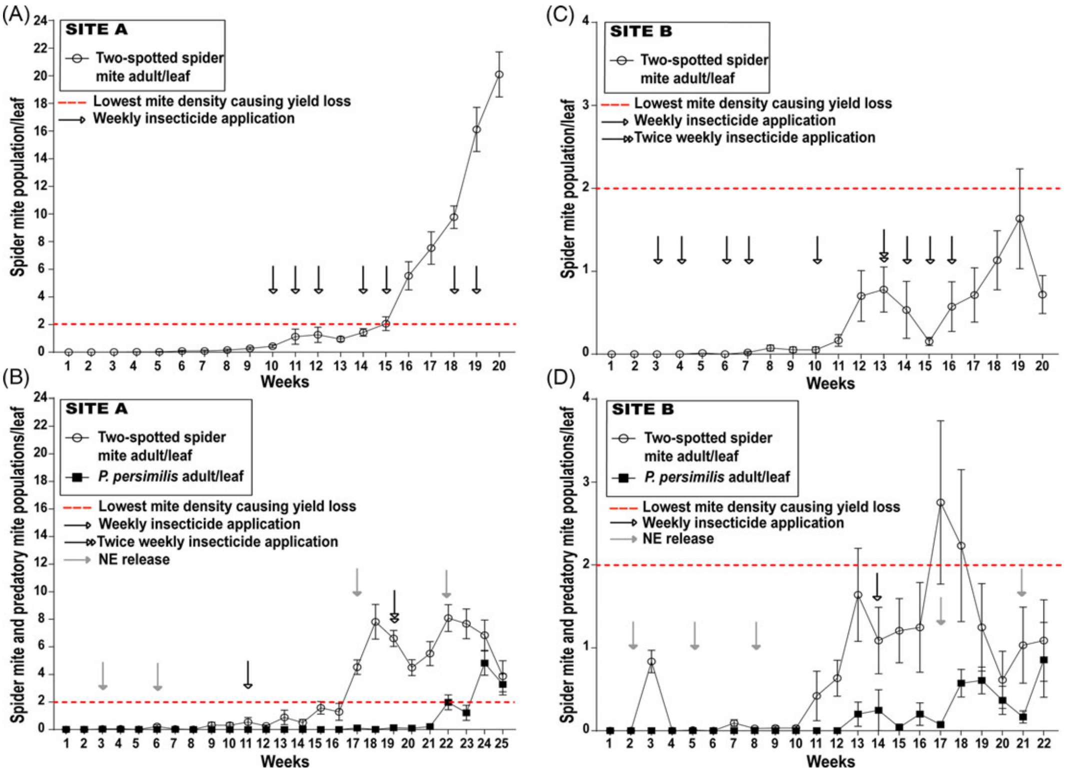

Fig. 4. The average number of two-spotted spider mite adults and *Phytoseiulus persimilis* adults on cucumber leaves in the integrated pest management and control greenhouses at sites A and B. **A**, Two-spotted spider mite adult population per cucumber leaf in the control greenhouse at site A; **B**, two-spotted spider mite adult and *P. persimilis* adult populations per cucumber leaf in the integrated pest-managed greenhouse at site A; **C**, two-spotted spider mite adult population per cucumber leaf in the control greenhouse at site B; **D**, two-spotted spider mite adult and *P. persimilis* adult populations per cucumber leaf in the integrated pest-managed greenhouse at site B. The error bars indicate the standard error of mean. Natural enemy releases (NE; grey arrows), weekly two-spotted spider mite targeting acaricide sprays (black arrows), and spider mite population density at which 23.8% cucumber yield loss is recorded (dashed red line).

steadily, reaching a peak of 20 two-spotted spider mites per leaf. Thus, the farmer was forced to stop production in the control greenhouse at 20 weeks post-transplanting (Fig. 4A). In the integrated pest management greenhouse, three *P. persimilis* releases and three acaricide sprays with minimal side effects on *P. persimilis* contributed to the reduction of the two-spotted spider mite population to four two-spotted spider mites per leaf at the end of the growing season (Fig. 4B). A significant moderate positive correlation was recorded between the two-spotted spider mite and the *P. persimilis* populations, with a peak of four *P. persimilis* mites per leaf ($r = 0.649$, $P = 0.0004$; Table 3). The mean numbers of two-spotted spider mites in both the integrated pest management and control greenhouses did not significantly differ ($U = 175.5$, $P = 0.516$; Table 2). In addition, a nonsignificant weak positive correlation was recorded between the *P. persimilis* and *A. swirskii* populations in the integrated pest management greenhouse ($r = 0.391$, $P = 0.585$; Table 3).

Site B. In the site B control greenhouse, 10 acaricide sprays maintained the two-spotted spider mite population below two spider mites per leaf throughout the growing period (Fig. 4C; Table 3). In the integrated pest management greenhouse, three predatory mite releases and one acaricide application with minimal side effects on *P. persimilis* maintained the two-spotted spider mite population below two spider mites per leaf throughout the growing period (Fig. 4D). A significant strong positive correlation was recorded between the two-spotted spider mite and the *P. persimilis* populations ($r = 0.788$, $P < 0.0001$). The mean number of two-spotted spider mites recorded on cucumber leaves did not significantly differ between the integrated pest management and control greenhouses ($U = 161$, $P = 0.295$; Table 2). In addition, there was no evidence of correlation between the two predatory mite populations ($r = 0.010$, $P = 0.949$; Table 3).

Discussion

This study aimed to assess the efficacy of integrated pest management measures based mainly on biological control that was complemented with chemical sprays only when deemed necessary for the control of whitefly, thrips, and two-spotted spider mite populations in cucumber greenhouse production, as compared to the efficacy of conventional chemical pest control strategies. The growers applied insecticidal and acaricidal sprays at the selected study sites at weekly or 10-day intervals to suppress pest populations and to reduce crop damage. Our study, conducted under the growers' routine production operations at each site, shows that the predatory mites effectively control cucumber pest populations when coupled with additional integrated pest management measures, including but not limited to preventive measures and periodic monitoring. In addition, we show that, depending on the pest and predator species, the integration of biological control agents for cucumber pest management is comparable to, or more effective than, the application of numerous insecticidal and acaricidal sprays throughout a growing season.

As a first step towards meeting this study's objective, we selected appropriate natural enemy organisms to control the region's major cucumber pest populations. The efficacy of *A. swirskii* mites in suppressing whitefly and thrips populations on diverse crops (Messelink *et al.* 2008; Calvo *et al.* 2011, 2015) and their origin in the Mediterranean region made this predatory mite suitable for use in our study. *Phytoseiulus persimilis* was chosen as a predatory mite for two-spotted spider mites because of its reported effectiveness in controlling *T. uritcae* in diverse crop management systems (McMurtry *et al.* 2013). Because *A. swirskii* was reported to prey on *P. persimilis* nymphs, larvae, and eggs, there was concern regarding intraguild predation between the predatory mites. Although intraguild predation may constitute a problem under low prey availability (Haghani *et al.* 2015; Maleknia *et al.* 2016), the present study shows that monitoring pest populations and releasing *A. swirskii* mites according to the availability of their preferred food sources, whiteflies and thrips, allowed for the simultaneous introduction of *A. swirskii* and

P. persimilis. This observation was supported by the absence of a significant correlation between the populations of both predatory mites in the integrated pest management greenhouses at both study sites. This was further supported at site B, where towards the end of the growing season, the population of *P. persimilis* increased in the presence of *A. swirskii*.

Temperature and humidity may have diverse effects on both pest and predatory mite population dynamics. The lower temperature threshold of *A. swirskii* development is 11.3 °C, the upper temperature threshold is 37.4 °C, and an optimum development temperature is 31.5 °C (Lee and Gillespie 2011). Similarly, *P. persimilis* has optimum development at 27 °C. However, Steneth (1979) showed that *P. persimilis* efficiently controls *T. urticae* at temperatures ranging from 15 °C to 27 °C. The selected study sites in the present study differed in elevation and latitude, with the average temperature at site A being 2.4 °C lower than that at site B, whereas the average relative humidity at sites A and B was similar, at 67.3 and 67.5%, respectively. In addition, the present study's experiments were conducted during winter and spring, when mean temperature ranged below the optimum development temperature of both predatory mites (5.8 °C to 26.2 °C and 12.18 °C to 24.3 °C, sites A and B, respectively). Because of this, multiple predatory mite introductions were required to control the pest populations in the integrated pest management greenhouses.

As a second step towards meeting our study objective, we determined the potential of *A. swirskii* in the simultaneous suppression of whiteflies and thrips on cucumber plants, as compared to the efficacy of chemical pest control. Our data show that, when no whitefly- and thrip-targeting insecticides were used, *A. swirskii* suppressed whitefly and thrips populations. At both sites (A and B), *A. swirskii* maintained the whitefly and thrips populations below the economic threshold of 4.6 whiteflies per cucumber leaf (Shen *et al.* 2005), 1.3 thrips per cucumber leaf (Steiner 1990), and 3–5 thrips per cucumber flower (Shipp *et al.* 2000), whereas in the control greenhouses, five insecticide sprays targeting whitefly and thrips at site A and 12 such sprays at site B were needed to maintain thrips and whitefly populations below the economic threshold. Our observation agrees with studies reporting the success of biological control agents, including *A. swirskii*, in controlling greenhouse whitefly populations, as compared to insecticide sprays (Stansly *et al.* 2004; Rodríguez *et al.* 2019). Although *A. swirskii* mites' ability to suppress the whitefly and thrips populations on cucumber plants is well documented (Nomikou *et al.* 2002, 2003; Messelink *et al.* 2005, 2006, 2008; Calvo *et al.* 2011), the efficacy of the mites' pest population suppression had not been compared to widely used conventional chemical pest control strategies.

Likewise, we determined the potential of *P. persimilis* mites for managing two-spotted spider mite populations on cucumber leaves. Our results show that *P. persimilis* mites maintained two-spotted spider mite populations below two spider mites per leaf, the population density at the seventh- or eighth-leaf stage at which 23.8% yield damage is recorded (Atanassov 1997), when use of *P. persimilis* was coupled with three and one acaricidal sprays at sites A and B, respectively. Acaricides were sprayed in the integrated pest management greenhouse due to the delayed arrival of the *P. persimilis* stock and its damage during the shipment. In addition, we report a positive correlation between the populations of *P. persimilis* and the two-spotted spider mites, suggesting the establishment of *P. persimilis* in the integrated pest management greenhouse with two-spotted spider mite serving as a food source. Our data are supported by several studies showing the predation efficiency and oviposition rate of *P. persimilis* when fed a diet consisting of two-spotted spider mites (Masoud 2007; Gillian 2008; Moghadasi *et al.* 2016; Fathipour *et al.* 2017; Yanar *et al.* 2019; Ahmadi *et al.* 2020). In the control greenhouses at sites A and B, seven and 10 insecticides and acaricides were sprayed, respectively, to control two-spotted spider mite populations. However, in the site A control greenhouse, seven consecutive acaricide sprays failed to keep the two-spotted spider mite population below damaging levels, leading to the premature termination of production in the control greenhouse. Yanar *et al.* (2019) reported similar results in a screenhouse trial in Tokat province, Turkey, which showed higher control of two-spotted spider mite populations on cucumber plants by *P. persimilis* mites compared to conventional acaricide applications.

The present study provides initial insights into the efficacy of simultaneous introductions of two predatory mites to control three major greenhouse cucumber pests in Lebanon, a coastal Mediterranean region. The study also provides an understanding of the benefits of adopting integrated pest management measures compared to those of chemical pest controls. Our results show that *A. swirskii* and *P. persimilis*, when used with additional integrated pest management measures, are highly effective in suppressing whitefly, thrips, and two-spotted spider mite populations on greenhouse cucumbers in Lebanon. Adopting biologically based integrated pest management in the Mediterranean region would yield considerable health benefits to consumers, farmworkers, and the environment by mitigating the negative health hazards of large numbers of pesticide sprays. However, crucial considerations should be highlighted: (1) proper scouting and timing of predatory mite introductions in the greenhouse for efficient pest control are necessary; (2) abiotic factors, including temperature and humidity during the growing season, which might impact predatory mite population dynamics, need to be considered; (3) disease-resistant plant cultivars that reduce pesticide applications and thereby reduce their negative impact on predatory mites should be chosen; and (4) the relatively high cost of international transport of natural enemies as well as damages that may occur during transportation can affect the application of biological control agents. Therefore, local production of natural enemies should be encouraged to eliminate transportation and customs costs and to make biocontrol agents readily available, as needed, to vegetable growers.

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